Theriogenology 84 (2015) 237–241

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Corpus luteum blood flow evaluation on Day 21 to improve the management of embryo recipient herds

C.R.B. Guimarães [a], M.E. Oliveira [b], J.R. Rossi [b], C.A.C. Fernandes [b], J.H.M. Viana [c], M.P. Palhao [b,*]

[a] Department of Animal Science, José do Rosário Vellano University (Unifenas), Alfenas, Minas Gerais, Brazil
[b] Department of Veterinary Medicine, José do Rosário Vellano University (Unifenas), Alfenas, Minas Gerais, Brazil
[c] Embrapa, Juiz de Fora, Minas Gerais, Brazil

ARTICLE INFO

*Article history:*
Received 26 November 2014
Accepted 10 March 2015

*Keywords:*
Color doppler
Timed embryo transfer
Diagnosis of pregnancy

ABSTRACT

The aim of the present study was to use blood flow evaluation of the CL at 14 days after embryo transfer to detect nonpregnant animals and optimize the management of bovine recipients. The estrous cycle was synchronized in 165 recipients, and the day of expected ovulation was considered to be Day 0. Embryo transfer was performed 7 days later, on Day 7. On Day 21, pregnancy was diagnosed on the basis of blood flow evaluation of the CL (DG21—predictive diagnostic). To validate this methodology, visual scores for blood flow were compared to objective data extracted from CL ultrasound images recorded in the Doppler mode. The size was also evaluated using recorded images of the CL in the B mode. Blood samples were also collected for further analysis of the progesterone (P4) concentration. The diagnosis of pregnancy was confirmed at 35 days after estrus (DG35—definitive diagnostic). The DG21 showed that 55.2% (90 of 163) of the animals were presumptively pregnant, and this value was higher ($P < 0.04$) than that obtained at DG35 (43.6%, 71 of 163). The predictive diagnostic achieved moderate specificity (79.3%) for the detection of pregnancy, but most importantly, high sensitivity (100%) for the detection of nonpregnant recipients. The overall accuracy of the diagnosis was 88.3%. The P4 concentrations were different ($P < 0.02$) and correlated with each visual score assigned for the CL size. Visual scores for CL blood flow were also efficient ($P < 0.0001$) to distinguish animals with different levels of P4; however, P4 concentrations were higher for scores 1 and 2 (high and regular blood flow, respectively) than those for score 3 (low blood flow). This technique showed high sensitivity and facilitated the early detection of nonpregnant animals. The DG21 would allow about 79.3% of nonpregnant animals to be resynchronized 9 to 14 days earlier, when compared to conventional management based on pregnancy diagnosis at Days 30 to 35.



## 1. Introduction

The management of recipients is a major cost in embryo transfer (ET) programs. In well-managed herds, obtaining large numbers of pregnancies in a short period of time is desirable. Thus, the prompt identification of nonpregnant animals and the preparation of these animals for new ET are highly important for the rationale use of bovine recipients.

The diagnosis of pregnancy in B-mode ultrasonography is based on the visual identification of the gestational vesicle. The results of this technique are based on the volume of the vesicle, and high precision is achieved when the diagnostic is performed after 25 days of gestation [1].

* Corresponding author. Tel.: +55 35 3299 3000; fax: +55 35 3299 3101.
*E-mail address:* millerpalhao@yahoo.com.br (M.P. Palhao).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.03.005

Currently, diagnosis based on the blood flow evaluation of the CL enables the CL from nonpregnant animals to be distinguished, after the expected period of maternal recognition, using color Doppler ultrasonography [2].

The CL is a highly vascularized gland responsible for progesterone (P4) secretion during the luteal phase of the estrous cycle and extending throughout the gestational period in cattle. The transition to the gestational CL involves the inhibition of luteolysis and the maintenance of the luteotrophic stimulus [3]. The vascularization of the gland evaluated through color Doppler ultrasonography is positively correlated with P4 secretion [4,5]. Thus, variations in blood flow observed between pregnant and nonpregnant females from 15 to 18 days after artificial insemination (AI) could be indicative of luteolysis in nonpregnant animals; however, individual effects do not ensure high reliability of the diagnosis of pregnancy performed at this time [6]. However, during the transition from one estrous cycle to the next, approximately 20 to 21 days after AI, the evaluation of the CL blood flow facilitates the precise identification of nonpregnant animals [2].

Thus, the objective of the present study was to evaluate blood flow in the CL at 14 days after ET to detect nonpregnant animals and improve the management of bovine recipients.

## 2. Materials and methods

This study was conducted in the south of Minas Gerais (Brazil) using 165 crossbred embryo recipients, heifers and cows (2–5 years) in good body condition score (3.5 ± 0.5, range 1–5 [7]). The recipients were maintained in an outdoor grazing system (*Brachiaria decumbens*), with free access to water and mixture supplement containing minerals and vitamins. All animals were cycling with no reproductive abnormalities detected in gynecologic exams and were previously immunized against bovine viral diarrhea, bovine herpesvirus type I, and *Leptospira*. All experimental procedures were previously approved by the Ethics for Animal Use Committee of the University of Alfenas—Unifenas (Protocol CEUA-Unifenas 23A/2012).

### 2.1. Synchronization protocol

The recipients were synchronized using the hormone protocol for the timed ET. The expected day of ovulation was defined as experimental Day 0. At 11 days before ovulation (Day−11), the animals received an intravaginal P4 (Prociclar, 0.75 g; Hertape Calier, Juatuba, Minas Gerais, Brazil) device and an intramuscular injection of 2-mg estradiol benzoate (Benzoate HC; Hertape Calier). Eight days later (Day−3), the intravaginal device was removed and an intramuscular injection of 150 μg of (D+) sodium cloprostenol (Veteglan Luteolícito, Hertape Calier) was administered. On Day 2, a 1-mg injection of estradiol benzoate was administered at the conclusion of the protocol.

### 2.2. Experimental design

Seven days after the expected ovulation (Day 7), only those animals with a good quality of CL, grade 1 or 2 assigned on the basis of the size score evaluated through transrectal palpation [8], received a cryopreserved (ethylene glycol) embryo of Angus breed. The embryos were at the same development stage (blastocyst) and grade 1 quality (Embryo Plus, Brits, South Africa). Transcervical ET was performed in the uterine horn ipsilateral to the ovary bearing a CL. Fourteen days after ET, the predictive diagnostic of nonpregnant recipients (DG21) was performed through visual assessment (the same technician evaluated the blood flow characteristics of the CL adjusted in scores). Additional scores for the size assessment of the CL were also obtained. Video records containing the entire cross section of the CL in B- and color Doppler modes were stored for subsequent image analysis and the validation of scores for blood flow and CL size. The animals were retrospectively classified into pregnant or nonpregnant groups. In addition, a blood sample was collected for further analysis of the P4 levels. On Day 35, approximately 28 days after ET or 14 days after DG21, pregnancy was diagnosed (DG35) on the basis of visual identification of the embryonic vesicle to confirm the DG21.

### 2.3. Score rate on the basis of size and blood flow of the CL and DG21

On Day 21, after the entire view of the CL cross section in B mode, the same technician assigned scores on the basis of size: 1 (large), 2 (averaged), or 3 (small CL). In the color Doppler mode, the same cross section of the CL was scanned, and visual scores were assigned on the basis of color signs covering the gland: 1 (high), 2 (regular), or 3 (small blood flow signs). The predefined criteria for blood flow scores included the presence of Doppler color signs at the border of the gland and within the luteal tissue. The CL assigned a score of 1 had blood flow at the border and within the luteal tissue well distributed along the cross section. A score of 2 was also associated with blood flow along the border and within the luteal tissue but restricted to particular parts of the cross section. The CL was designated a score of 3 when small signs of the blood flow were observed in the periphery and restricted to few parts of the gland.

The predictive diagnostic (DG21) of nonpregnant was designed for recipients with a score of 3 for luteal blood flow. For animals designated as “pregnant”, the CL in the same ovary as reported on Day 7 was designated with a blood flow score of 1 or 2.

### 2.4. Measurements of CL (ultrasound, software, and storage unit)

The same technician performed all examinations using the same ultrasonographic device (Mindray M5). In the B mode, the machine was preset to a frequency of 6.5 MHz, with 32 frames per second, gain of 72, 100% power, a 6.1-cm image depth, and a 2-cm focal point (where most of the time the ovary was positioned on the screen). In color Doppler mode, the settings were adjusted for a velocity range of 6.0 cm/s to detect blood cell movements in small vessels [9]. For this purpose, a frequency of 4.2 MHz, with

15 frames per second, 100% power, gain of 58, and a pulse frequency of 0.6 kHz (0.6 PRF).

Videos containing the entire cross section of the CL in B (approximate sequence of 400 frames) and color Doppler (approximately 100 frames) modes were saved onto an external HD (Samsung 500 GB) in .avi format. Videos in the B mode were further analyzed using VirtualDub software (version 1.5), and a CL image at the maximum diameter was extracted and saved in .jpeg format. Subsequently, the image was transferred to ImageJ (Image Processing and Analysis in Java) software and, after adjusting the scale, the total area ($mm^2$) was indicated using the cursor surrounding the entire circumference of the CL.

The videos in Doppler mode were similarly analyzed to calculate the area of colored pixels indicating blood flow signs from the CL. For further calculation of the CL blood flow area, a weighted average (according with the size on each section) of three distinct regions of the CL (central portion and opposite side) was considered. This methodology was adopted because the blood flow did not follow a specific pattern along the luteal structures [10]. Portions of the gland have different blood signs, and analyzing images from one part of the gland could overcome the underestimation or overestimation of the luteal blood flow. Data from the CL measurements were then used to validate the visual evaluation.

In case of new ovulation, small luteal structure with intense blood flow signs was observed in the same or the opposite ovary of the CL reported on Day 7 and, the animal was considered non-pregnant. Thus, data from area and blood flow of the CL were assembled as 0 for statistical analysis.

### 2.5. Blood collection and determination of progesterone

Blood samples were collected through puncture of the coccygeal vein or artery using a multiple 21-ga needle, and the samples were stored in heparinized vacuum tubes at approximately 4 °C. The samples were further centrifuged at 600 × *g* for 10 minutes. Plasma was recovered using an automatic pipette and stored at −20 °C for the subsequent determination of P4 concentrations. The measurements were obtained through RIA using a commercial kit according to the manufacturer's instructions (Immunotech SAS, Marseille, France). The sensitivity of the assay was 0.05 ng/mL (0.16 nmol/L), and the intra-assay coefficient of variation was 6.5%. Quality control was performed according to the manufacturer's instructions using samples with known concentrations of P4.

### 2.6. Statistical analyses

The sensitivity, specificity, and accuracy of the pregnancy diagnosis resulted from the potential arrangements of predictive (DG21) and definitive (DG35) diagnostic tests: (1) DG21 correct positive, (2) DG21 false-negative, (3) DG21 false-negative, and (4) DG21 correct-negative (Table 1). The sensitivity of the diagnostic was calculated using the formula: $[a/(a+c)] \times 100$, and the specificity was calculated as $[d/(b+d)] \times 100$. Positive (PPV) and negative predictive values (NPV) were calculated as PPV = a/(a + b) and NPV = d/(c + d), and the overall accuracy (ACC) of the examination was defined as ACC = (a + d)/(a + b + c + d).

**Table 1**
Frequency distribution of pregnant and nonpregnant recipients on the basis of predictive (DG21) and definitive (DG35) diagnostics.

| Frequency of the results | DG35—visual identification of the embryonic vesicle | | Total |
|---|---|---|---|
| | Positive | Negative | |
| DG21—presence and blood flow evaluation of the CL | | | |
| Positive | 71 (a) | 19 (b) | 90 (a + b) |
| Negative | 0 (c) | 73 (d) | 73 (c + d) |
| Total | 71 (a + c) | 92 (b + d) | 163 |

Sensitivity = ([a/(a + c)] × 100) = 100%.
Specificity = ([d/(b + d)] × 100) = 79.3%.
Positive predictive value = a/(a + b) = 78.9%.
Negative predictive value = d/(c + d) = 100%.
Accuracy = (a + d)/(a + b + c + d) = 88.3%.

The variables generated after measuring the total and blood flow area of the CL, and the P4 concentrations were analyzed using the Kruskal–Wallis test. The statistics of this test were used to compare the means between the groups defined according to the pregnancy diagnosis (pregnant and nonpregnant). Differences among mean group scores based on the size and blood flow of the CL (1, 2, and 3) were assessed using Tukey's test. Probabilities lower than 5% were considered statistically significant.

## 3. Results

On DG21, a new CL on the opposite side of the ovary to that recorded on Day 7 was only detected in six animals (3.6%, 6 of 165) considered nonpregnant, and this diagnosis was subsequently confirmed on DG35. Furthermore, the presence of new ovulation in the same ovary occurred in 2.4% (4 of 165) of the cases. The new luteal tissue was classified as a small-sized CL with regular or good blood flow (score 1 or 2). Previous knowledge of the ovarian side and the size of the CL reported on Day 7 led to a nonpregnant diagnosis, which was subsequently confirmed on DG35. Additionally, two animals scoring 2 for CL blood flow, which would normally be diagnosed as pregnant, had some uterine contents compatible with uterine infection and were excluded from statistical analysis.

The pregnancy rate observed for the diagnostic based on the presence and blood flow evaluation of the CL (DG21) was 55.2% (90 of 163), and this value was higher ($P < 0.04$) than that observed 14 days later (DG35; 43.6%, 71 of 163). Comparing to conventional pregnant diagnosis performed here on Day 35, 79.3% (73 of 92) of nonpregnant animals were detected on the DG21. The predictive diagnostic showed high sensitivity (100%) for the detection of all pregnant recipients and moderate specificity (79.3%) for the determination of negative animals. However, an NPV of 100% reflected no false-negative results. The ACC of the diagnostic performed at 14 days after ET (DG21) and based on the blood flow evaluation of the CL was 88.3% (Table 1).

The predictive scores used in the present study to classify the total and blood flow areas of the CL were validated through measurements obtained from images in the B and color Doppler modes, respectively (Table 2).

Significant differences in the measurements of each score validated the visual criteria used to separate the CL according to the size and blood flow.

Furthermore, visual scores of size and blood flow were used to compare circulating P4 on Day 21 (Table 3). The levels of plasma P4 were different ($P < 0.02$) for recipients grouped in each of the visual scores assigned for CL size. The predictive assessment of the CL blood flow, however, only distinguished two levels of P4. The recipients with CL designated with scores 1 and 2 (high and regular blood flow, respectively) had higher ($P < 0.0001$) concentrations of P4 than recipients with CL scores of 3 (low blood flow).

The CL blood flow is an adequate indicator of pregnancy performed on Day 21; nonpregnant recipients show lower ($P < 0.0001$) blood flow areas of the CL compared with pregnant animals (Table 4). The clear difference between scores 2 and 3 (Table 2) is that the blood flow area is approximately 15 times greater in CL with a score of 2, and this difference simplifies the detection of CL with low blood flow. The evaluation of the CL size alone is a good indicator of pregnancy on Day 21. Nonpregnant animals have a smaller CL ($P < 0.0001$) compared with pregnant animals (Table 4). However, the operator has a small rate of error between scores 2 and 3 (Table 2) for predictions based on the size of the CL.

## 4. Discussion

The prompt identification of nonpregnant animals within a group of recipients is needed to reduce the days available for new ET, thereby optimizing the production system. In the present study, the detection of nonpregnant animals was based on the evaluation of the blood flow in the CL at 14 days after ET.

For practical application, the performance time is extremely important, considering the use of diagnostic methodology in routine practice for reproductive management [2]. Here, information concerning the previous location of the CL (ET on Day 7) increased the accuracy of the diagnosis and reduced the time required. Visual evaluations at 14 days after the ET were performed in 10 to 15 seconds because only the ovary containing the CL could be further evaluated on DG21.

The DG21 confirmed the ability to detect nonpregnant animals (NPV = 100%), supporting the need for the early diagnosis of pregnancy [2,11,12]. The detection of pregnant animals was also high (PPV = 78.9%). The primary factors implicated in false-positive diagnostics (21.1%, 19 of 90) are the occurrence of long estrous cycles, when the CL remains active for more than 21 days [13], early embryonic losses occurring between DG21 and DG35 [4,14,15], and short estrous cycles (9–10 days) with the ovulation in the same ovary [16].

Variations in the CL blood flow initiated between 15 and 18 days of the estrous cycle indicate the beginning of the luteal regression [4,6]. However, during this period, the diagnosis of pregnancy based on the CL blood flow evaluation does not ensure high reliability, primarily reflecting individual variations [6]. Thereafter, sequential evaluations of the CL blood flow in embryo recipients showed an increase in the specificity of the diagnostic performed between 17 and 19 days of the estrous cycle (Day 19 = 54.3%, [15]). The conclusion was that the assessment of CL blood flow alone was insufficient for diagnosis. However, these studies focused on the correct diagnosis of pregnancy (positive and negative) and the high accuracy of the examination.

During the transition period between cycles at 20 to 21 days after AI, the diagnosis based on blood flow evaluation of the CL facilitated the precise identification of nonpregnant animals in a single examination [2]. In the present study, the adapted methodology was used to evaluate embryo recipients at 14 days after ET, and the previous information concerning the CL location and the validation of scores resulted in increased accuracy, PPV, and NPV (74.8%, 65.1%, and 98.5%, respectively, [2]). The specificity and sensitivity observed in the present study were higher than those previously reported for diagnoses performed at 12 days after TE (82.9% and 54.3%, respectively) [15]. No false-negative diagnoses were recorded when the technique was used in ET (this study) compared with 0.4% of tests used for the diagnosis of AI [2].

The CL blood flow was positively correlated with P4 secretion [4,5]. The results of the present study confirmed

**Table 2**
Total and blood flow areas ($mm^2$) of the CL in each of the groups designated according the subjective scores assigned for the size and blood flow of the CL.

| Score | CL size (B mode) | | CL blood flow (Color Doppler) | |
|---|---|---|---|---|
| | n | Area | n | Area |
| 1 | 50 | $345.0 \pm 54.9^{a}$ | 70[d] | $69.8 \pm 24.0^{a}$ |
| 2 | 42 | $262.1 \pm 67.5^{b}$ | 19 | $47.3 \pm 22.2^{b}$ |
| 3 | 71 | $102.8 \pm 60.0^{c}$ | 73 | $3.1 \pm 4.2^{c}$ |
| Total | 163 | | 162 | |
| P value | | <0.0001 | | <0.0001 |

[a,b,c]Means with different letters in the same column are different according to Tukey's test.
[d] Doppler scanner of the CL was not recorded for one recipient.

**Table 3**
The progesterone concentrations (ng/mL) in each of the groups designated according to the subjective scores assigned for the size and blood flow of the CL.

| Score | CL size | | CL blood flow | |
|---|---|---|---|---|
| | n | Progesterone | n | Progesterone |
| 1 | 38 | $3.3 \pm 1.6^{a}$ | 55 | $3.0 \pm 1.5^{a}$ |
| 2 | 34 | $2.3 \pm 1.8^{b}$ | 15 | $2.8 \pm 2.1^{a}$ |
| 3 | 51 | $0.4 \pm 0.7^{c}$ | 53 | $0.3 \pm 0.6^{b}$ |
| P value | | <0.02 | | <0.0001 |

[a,b,c]Means with different letters in the same column are different according to Tukey's test.

**Table 4**
Total and blood flow areas ($mm^2$) of the CL in each of the groups designated according to the predictive diagnosis of pregnant or nonpregnant animals on DG21.

| Diagnosis | DG21 | | DG21 | |
|---|---|---|---|---|
| | n | Total area | n | Blood flow area |
| Pregnant | 90 | $312.3 \pm 64.3^{a}$ | 89[c] | $65.0 \pm 25.3^{a}$ |
| Nonpregnant | 73 | $102.1 \pm 60.9^{b}$ | 73 | $3.1 \pm 9.9^{b}$ |

[a,b]Means with different letters in the same column are different ($P < 0.0001$) according to the Kruskal–Wallis test.
[c] Doppler scanner of the CL was not recorded for one recipient.

that the plasma P4 concentrations were coherently associated with predictive diagnostics (positive or negative). The P4 concentrations differed between active (scores 1 and 2) and inactive (score 3 for blood flow) structures. This feature suggested the adoption of only two scores (positive and negative) for the visual identification of the CL blood flow. However, the visual evaluation of the CL size was more consistent with plasma P4 concentrations. The plasma P4 concentration is strongly correlated with the area and volume of luteal tissue [17]. This information is important because, apparently, the scores assigned for CL size could indicate pregnancy. In the present study, for example, if the recipients with a CL size score of 3 were considered nonpregnant, then, the ACC of the test would fall to 82.8%. The actual accuracy is higher than results obtained on the basis of the visualization of the uterine contents between Days 21 and 25 (65.1% of correct diagnoses [18], on Day 21 [68.8%] [12], and similar to analyses performed on Day 22 [82.9%] [12]). However, in the present study, the diagnosis of pregnancy based only on the visual identification of the CL size showed an NPV of 94.4% and four false-negative diagnoses; a CL classified as small (score 3) and subsequently identified as pregnant (DG35) was observed. Apparently, Doppler sonography is more sensitive for the discrimination of positive and negative recipients, with a CL blood flow area approximately 15 times greater for a score of 2 (positive) compared with a score of 3 (nonpregnant).

Several aspects are considered to improve the accuracy of the diagnosis of pregnancy on Day 21: the preset of the equipment to detect the movement of red blood cells in small ovarian vessels [9] and the adjustment of the pulse frequency to 0.6 kHz (PRF = 0.6), possible interference of the local power supply or animal motion, and the experience of the technician in performing the measurement and interpreting the generated images.

This technology facilitates the rational use of the recipients in ET programs. The DG21 anticipates the diagnosis of pregnancy and increases the number of receptors available for further timed ET protocols. Typically, the reproductive management of recipients is based on diagnosis through the visualization of the embryonic vesicle [1]. Thus, less intensive management considers the beginning of the resynchronization protocol at the time of diagnosis (Days 30 to 35) and new ET after approximately 17 days (Day 47). Using the DG21, nonpregnant recipients could be resynchronized 9 to 14 days earlier. In a new situation, the DG21 facilitates the beginning of the protocol at 1 week after the ET (on Day 13, for example), reducing 17 to 22 days of the service period. Furthermore, considering a gestation period of 280 days, a gain of 17 days would provide an additional calving for every 17.

### 4.1. Conclusions

The visual evaluation of the CL blood flow at 14 days after the ET is effective for the detection of nonpregnant recipients, without false-negative results. Information from ovarian assessment (on Day 7) and the validation of visual scores for CL blood flow improved the accuracy and positive and negative predictive values of the examination. This methodology increases flexibility in the use of recipients, allowing about 79.3% of nonpregnant animals to be resynchronized 9 to 14 days earlier.

## Acknowledgments

This study was financially supported by FAPEMIG Project CVZ-APQ 01454/12 and Biotran LTDA.

## References

[1] Ginther OJ. Ultrasonic imaging and animal reproduction. In: Pregnancy: ultrasonic anatomy and research. Book 3, Cattle. Cross Plains, WI: Equiservices Publishing; 1998. p. 131–78.
[2] Siqueira LGB, Areas VS, Ghetti AM, Fonseca JF, Palhão MP, Fernandes CAC, et al. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. J Dairy Sci 2013;96:6461–72.
[3] Salles MGF, Araújo AA. Cyclic and gestational corpus luteum: review. Revista Brasileira de Reprodução Anim 2010;34:185–94.
[4] Ginther OJ, Silva LA, Araujo RR, Beg MA. Temporal associations among pulses of 13,14-dihydro-15-keto-PGF2α, luteal blood flow, and luteolysis in cattle. Biol Reprod 2007;76:506–13.
[5] Herzog K, Brockhan-Lüdemann M, Kaske M, Beindorff N, Paul V, Niemann H, et al. Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology 2010;73:691–7.
[6] Herzog H, Voss C, Kastelic JP, Beindorff N, Paul V, Niemann H, et al. Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. Theriogenology 2011;75:549–54.
[7] Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989;72:68–78.
[8] Leal LS, Oba E, Fernandes CAC, Filho OGS. Corpus luteum evaluation, uterine contractility, plasma concentrations of progesterone and estradiol in bovine embryo recipients. Ciência Anim Brasileira 2009;10:174–83.
[9] Ginther OJ. Ultrasonic imaging and animal reproduction. Book 4. Color-Doppler ultrasonography. Cross Plains, WI, USA: Equiservices Publishing; 2007.
[10] Viana JHM, Arashiro EKN, Siqueira LGB, Ghetti AM, Areas VS, Guimarães CRB, et al. Doppler ultrasonography as a tool for ovarian evaluation. Proc XXVII Annu Meet Braz Embryo Transfer Soc (sbte) 2013; 27:112–118.
[11] Fricke PM, Lamb GC. Potential applications and pitfalls of reproductive ultrasonography in bovine practice. Vet Clin North Am Food Anim Pract 2005;21:419–36.
[12] Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszweski MA, Kraemer DC. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology 2006;66:1034–41.
[13] Giordano JO, Guenther JN, Lopes GJR, Fricke PM. Changes in serum pregnancy-associated glycoprotein, pregnancy specific protein B, and progesterone concentrations before and after induction of pregnancy loss in lactating dairy cows. J Dairy Sci 2012;95:683–97.
[14] Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvão KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim Reprod Sci 2004;82–83:513–35.
[15] Utt MD, Johnson GL, Beal WE. The evaluation of corpus luteum blood flow using color-flow Doppler ultrasound for early pregnancy diagnosis in bovine embryo recipients. Theriogenology 2009;71:707–15.
[16] Taponen J, Hjerppe P, Kopra E, Rodríguez-Martínez H, Katila T, Kindahl H. Premature prostaglandin F2alpha secretion causes luteal regression in GnRH-induced short estrous cycles in cyclic dairy heifers. Theriogenology 2003;60:379–93.
[17] Siqueira LG, Torres CA, Amorim LS, Souza ED, Camargo LS, Fernandes CA, et al. Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Anim Reprod Sci 2009;115:18–28.
[18] Pieterse MC, Szenci O, Willemse AH, Bajcsy CS, Dieleman SJ, Taverne MA. Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. Theriogenology 1990;33:697–707.